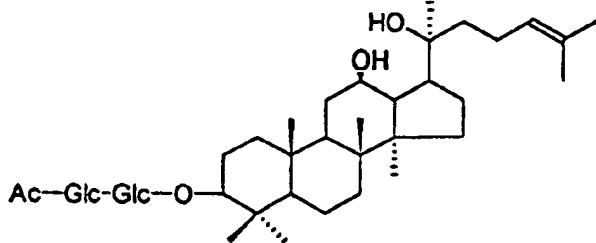
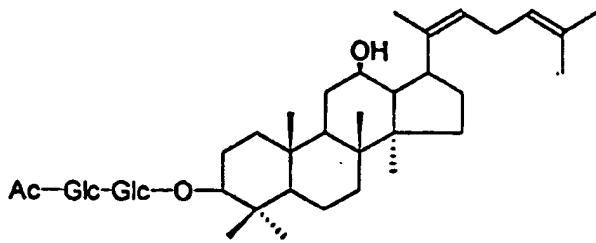




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(54) Title: NOVEL GINSENG SAPONIN COMPOUNDS, PROCESS FOR PREPARATION THEREOF AND ANTI-TUMOR AGENT COMPRISING THE SAME AS AN ACTIVE COMPONENT					
(57) Abstract					
<p>The present invention relates to novel ginseng saponin compounds having a potent anti-tumor activity, which are represented by formulae (I) and (II). The compounds (I) and (II) above are novel and can be produced by heating plants of ginseng genus for 0.5 to 20 hours at a high temperature of 110 to 180 °C, or can be synthesized by acetylating ginsenoside Rg3 and $\Delta^{20(22)}$-ginsenoside Rg3 which are known ginseng saponin compounds, respectively. The present invention also relates to an anti-tumor composition comprising these compounds (I) and/or (II) as an active ingredient.</p>					
 <p style="text-align: right;">(I)</p>					
 <p style="text-align: right;">(II)</p>					

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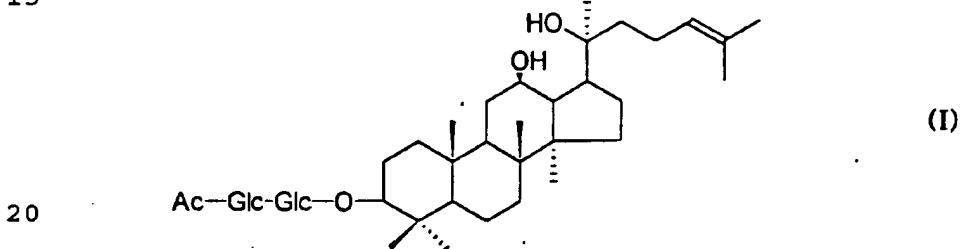
NOVEL GINSENG SAPONIN COMPOUNDS, PROCESS FOR PREPARATION THEREOF AND ANTI-TUMOR AGENT COMPRISING THE SAME AS AN ACTIVE COMPONENT

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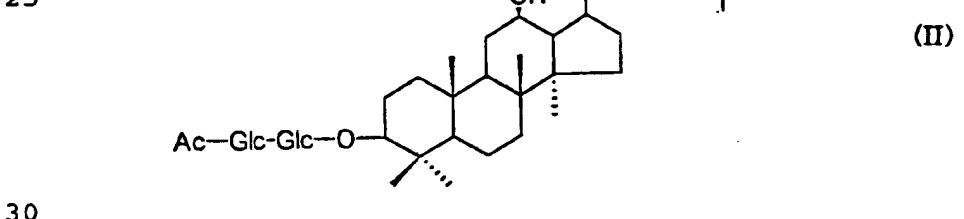
TECHNICAL FIELD

10 The present invention relates to novel ginseng saponin compounds having an anti-tumor activity. More specifically, the present invention relates to novel ginseng saponin compounds having a potent anti-tumor activity, which are represented by the following formulas (I) and (II).

15



25



The present invention also relates to a process for preparation thereof, and an anti-tumor composition comprising the same as an active component.

35

BACKGROUND ART

The physiological and biochemical researches in the
5 effect of ginseng, particularly red ginseng, have been
generally conducted on the subject of saponin components
which are contained in large quantities in ginseng and
has been known as the main component exhibiting the phar-
macological effect of ginseng. However, the minor sapo-
10 nin components contained in red ginseng merely in minute
quantities have been studied by very few groups heretofore
because they can be hardly separated from ginseng.

15 DISCLOSURE OF INVENTION

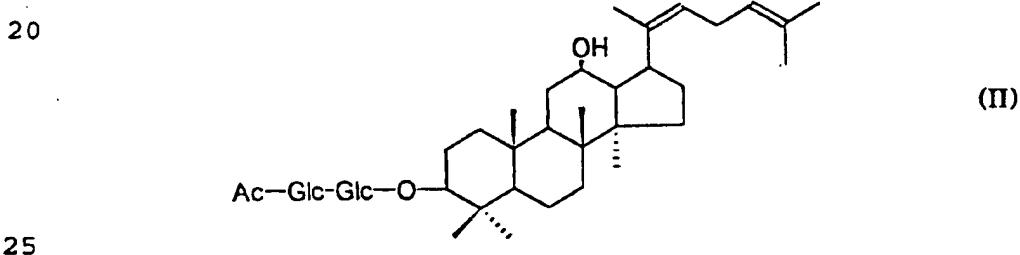
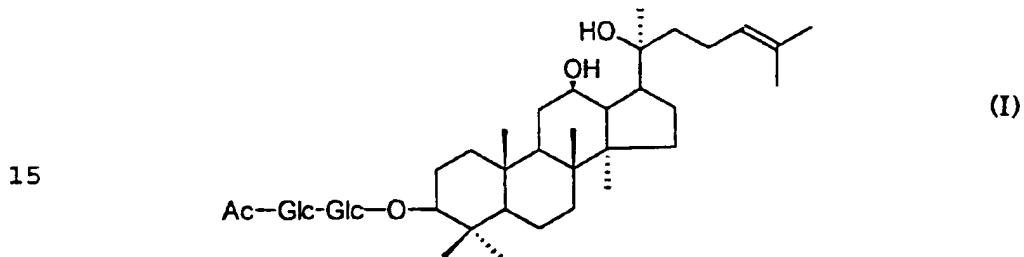
Thus, the present inventors have concentratedly stud-
ied to find a method for enhancing the pharmacological
20 effect of ginseng by treating ginseng under specific
conditions to increase the contents of specific components
and further for separating the respective components so
that the study of their pharmacological effect can be
made. As a result of such studies, we have identified
25 that when a ginseng is heat-treated for 0.5 to 20 hours at
a high temperature of 110 to 180°C, the contents of effec-
tive components which are present in a minor amount in
ginseng increase, and consequently a processed ginseng
having an enhanced pharmacological effect compared with
30 fresh ginseng, white ginseng or red ginseng is prepared.
In the procedure to determine the pharmacological effect
of the various components separated from the processed
ginseng, the present inventors have found novel components
which have never been disclosed heretofore, and subse-
35 quently identified the chemical structure, pharmacological
effect and process for preparation thereof. Thus, we
have completed the present invention.

Therefore, the present invention relates to saponin compounds identified as novel active components contained in ginseng.

5

The first object of the present invention is to provide the novel saponin compounds represented by the following formulas (I) and (II). The configuration at $\Delta^{20}(22)$ of (II) is zusammen or entgegen.

10



25

It is a further object of the present invention to provide the process for preparing the novel ginseng saponin compounds having the formulas (I) and (II) above.

30

Further, it is still another object of the present invention to provide an anti-tumor composition comprising the compound (I) and/or (II) as an active component.

35

BEST MODE FOR CARRYING OUT THE INVENTION

5 The compounds of formulas (I) and (II) according to
the present invention can be prepared by an extraction
from the processed product of plant of *Panax* genus or by a
synthetic method using known ginsenoside components as a
starting material.

10 First, according to the extraction method, roots or
leaves of the plant of *Panax* genus, for example, *Panax*
ginseng, *Panax notoginseng*, *Panax quinquefolium*, *Panax*
japonicus, etc., or tissue cultures thereof, or extracts
therefrom with water or lower alcohol are heated for 0.5
15 to 20 hours at a temperature of 110 to 180°C. The proc-
essed ginseng thus obtained is extracted with water, or a
suitable organic solvent, for example, lower alcohols such
as methanol, ethanol, etc., or a solvent mixture thereof,
and then the extract is concentrated under reduced pres-
20 sure, suspended in water and then extracted with a nonpo-
lar organic solvent such as hexane, ether, dichlorometh-
ane, chloroform, ethylacetate or a solvent mixture there-
of. The remaining aqueous layer is extracted with a polar
organic solvent such as butanol and then the extract
25 is subjected to chromatography to obtain a fraction con-
taining compounds (I) and (II). This fraction is crys-
tallized from a suitable solvent system, for example a
solvent mixture of water and lower alcohol, preferably a
solvent mixture of water and methanol in a ratio of 1:1 by
30 volume, to prepare the desired pure saponin compounds (I)
and (II).

According to this method, during the procedure of
heat-treatment of ginseng, a sugar moiety attached to the
35 20th carbon of panaxadiol saponins present in ginseng such
as ginsenosides Ra, Rb₁, Rb₂; Rc, Rd, etc. is removed and
an acetyl group is introduced into the 6th position of the

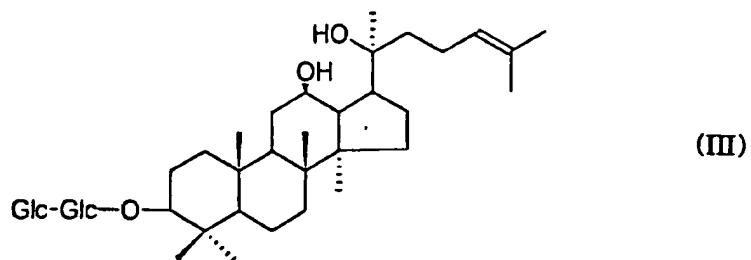
terminal glucose of the sugar moiety attached to 3rd carbon to produce the novel saponin compound (I). The saponin compound (I) can also be produced by removing the sugar moiety attached to the 20th carbon from ginsenosides 5 Rs_1 and Rs_2 . The saponin compound (II) is produced by removing the OH group attached to the 20th carbon and hydrogen at the 22th-position from the compound (I) through dehydration reaction. In this reaction, the stereochemical structure of the double bond at 20th-position can have cis or trans configuration.

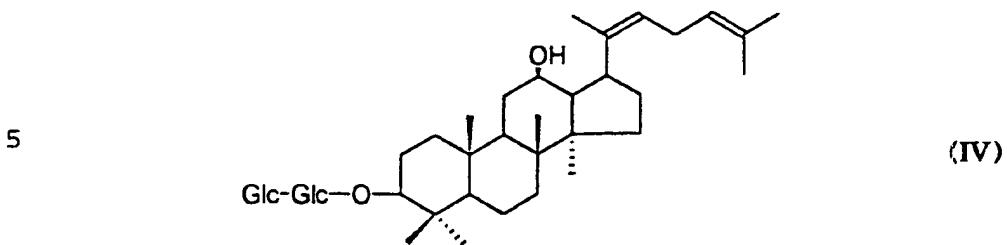
In the extraction method according to the present invention as mentioned above, the contents of the desired compounds (I) and (II) can be more increased by repeatedly 15 carrying out chromatography. In addition, if necessary, the order of the heat-treatment step and extraction step with organic solvent in this process can be inverted to obtain the same result.

20 According to the synthetic method of the present invention, the novel saponin compounds of formulas (I) and (II) can be obtained by acetyating the known ginsenoside compounds. Specifically, the compound of formula (I) can be produced by acetyating the known ginsenoside Rg_3 of 25 formula (III), and the compound of formula (II) can be prepared by acetyating the known $\Delta^{20(22)}$ -ginsenoside Rg_3 of formula (IV) which is formed by the dehydration reaction at 20th-position of ginsenoside Rg_3 .

30

35





10 Such acetylation reaction can be conducted using acetic anhydride(Ac_2O) or acetyl chloride as an acetylating agent. In this case, the acetylating agent can be used in a molar ratio of 1:1-4, preferably 1:1.2-2 with respect to the compound (III) or (IV). It is appropriate 15 to carry out this reaction at a temperature of -40°C to 20°C , preferably -40°C to 0°C , for 1 to 48 hours.

20 The novel saponin compound of formula (I) or (II) thus obtained can be further purified by a conventional working-up method, for example, selective crystallization, column chromatography, etc.

25 The novel saponin compounds (I) and (II) prepared according to the process of the present invention as mentioned above, have a potent anti-tumor activity, and therefore can be effectively used as an agent for prevention or treatment of cancerous disease such as hepatoma, 30 gastric cancer, leukemia, etc. Therefore, the present invention also relates to an anti-tumor composition comprising as an active ingredient the compound (I) or (II) or the mixture thereof.

35 When the composition containing the compounds (I) and/or (II) of the present invention is applied for clinical purposes as an anti-tumor agent, it can be combined with pharmaceutically acceptable carriers to prepare various formulations conventionally used in the pharmaceu-

tical field, for example, oral preparations such as tablets, capsules, troches, solutions, suspensions, etc.; injectable preparations such as injectable solutions or suspensions, or ready-to-use dried powder which can be 5 applied after re-constituted with injectable distilled water before injection, etc.; or locally applicable preparations such as ointments, creams, solutions, etc.

The carriers which can be used in the composition of 10 the present invention are conventional ones in the pharmaceutical field, for example, binders, lubricants, disintegrating agents, excipients, solubilizers, dispersing agents, stabilizers, suspending agents, coloring agents, flavors and the like in the case of oral preparations; 15 preservatives, agents for painlessness, solubilizers, stabilizers and the like in the case of injectable preparations; bases, excipients, lubricants, preservatives and the like in the case of locally applicable preparations. The pharmaceutical preparations thus produced can be 20 administered orally, or parenterally such as for example intravenously, peritoneally, subcutaneously, or can be topically applied. In addition, the oral preparations may be administered together with an antacid or in the form of an enteric-coated preparation which is formulated 25 by covering the orally administrable solid preparation such as tablet with the enteric coatings, in order to prevent decomposition of the preparation by gastric acid when it is administered per orally.

30 Although the administration dosage to a human being of the novel saponin compounds (I) and (II) according to the present invention can be selected depending on the absorption, inactivating rate and excretion rate of the active component in the body, age, sex and condition of the 35 subject patient, severity of the disorders to be treated and the like, it is generally administered to an adult in an amount of 5 to 500mg, preferably 10 to 200mg daily.

Therefore, when the composition of the present invention is formulated into the dosage unit form, each of the dosage unit form can contain the compounds (I) and/or (II) in an amount of 5 to 500mg, preferably 10 to 200mg on the 5 basis of the effective amount range as mentioned above. If necessary, the dosage unit form thus formulated can be administered using a specialized method according to the judgement of the specialist who arranges or observes the administration and the requirement of the individuals. 10 The total daily dosage can also be divided into several portions and administered over several times, preferably 1 to 6 times.

The present invention is more specifically explained 15 by the following examples and test examples. However, it should be understood that the present invention is not limited to those examples in any manner.

EXAMPLE 1 : Preparation of ginseng extract containing the
20 compounds (I) and (II)

100g of fresh ginseng was introduced into a sealed container and then heated for 2 hours at 130°C. The obtained processed ginseng was extracted with 200ml of 25 methanol to obtain the methanol extract and then methanol was removed from the extract by evaporation. The remaining residue was suspended in 100ml of water, extracted 3 times with 100ml of ether, and then the remaining aqueous layer was extracted 3 times with 100ml of butanol saturated with water to obtain the butanol extract containing 30 saponins. This butanol extract was dried and then subjected to silica gel column chromatography (eluent: ethyl acetate/methanol/ water = 20:1:1). According to the result of TLC analysis (developing solvent: ethyl acetate/methanol/ water = 10:1:1) of the eluates, 30mg of 35 a fraction containing 50% of the desired compound (I) having the Rf value of 0.25 and 25mg of a fraction con-

taining 55% of the compound (II) having the Rf value of 0.27 were obtained, respectively.

5 EXAMPLE 2 : Preparation of ginseng extract containing the compounds (I) and (II)

10kg of dried root-hair ginseng was extracted by refluxing with 20L of methanol for 4 hours. The ginseng extract thus obtained was dried under reduced pressure.
10 The resulting ginseng extract in the form of a syrup was introduced into an autoclave and then heated for 4 hours at 120°C. The heat-treated ginseng extract was subjected to silica gel column chromatography according to the same method as Example 1 and then the eluates were applied for
15 TLC analysis (developing solvent: ethyl acetate/methanol/water = 10:1: 1) to obtain 3g of a fraction containing 50% of the desired compound (I) having the Rf value of 0.25 and 2g of a fraction containing 60% of the compound (II) having the Rf value of 0.27, respectively.
20

Example 3 : Preparation of the compound (I)

1g of the fraction containing the compound (I) prepared in Example 2 above was subjected to silica gel column chromatography according to the same manner as Example 1 using the mixed solvent of ethyl acetate/methanol/water(20:1:1) as an eluent to obtain 400mg of a fraction containing 92% of the desired compound (I). The 30 fraction thus obtained was crystallized from the solvent mixture of methanol/water (1:1, v/v) to obtain 200mg of the desired compound (I).

The compound (I) thus obtained exhibits the following 35 physico-chemical characteristics :

Chemical name : 3 β ,12 β ,20 β -trihydroxy-damar-24-ene-3-O- β -

D-6"-O-acetyl-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside

Mass spectrum (FAB $^+$, m/z) : 827 ([M+H] $^+$), 849 ([M+Na] $^+$)
5 (FAB $^-$, M/Z) : 825 ([M-H] $^-$)

CNMR (δ ppm, pyridine-d5) : 16.1, 16.2, 16.7, 17.1, 17.4,
18.3, 20.9, 22.4, 22.7, 25.5, 26.3, 26.8, 27.7,
30.8, 30.9, 34.8, 35.4, 36.7, 39.1, 39.4, 39.8,
10 49.2, 50.1, 50.2, 51.6, 56.2, 62.5, 64.5, 70.7,
70.8, 71.1, 73.6, 75.0, 75.1, 77.6, 77.7, 78.2,
83.9, 88.9, 104.6, 105.8, 125.8, 130.5, 171.0

Example 4 : Preparation of the compound (II)

15 1g of the fraction containing the compound (II) prepared in Example 2 above was subjected to silica gel column chromatography according to the same manner as Example 1 using the solvent mixture of ethyl acetate/methanol/water(20:1:1) as an eluent to obtain 300mg of a fraction containing 95% of the desired compound (II). The fraction thus obtained was crystallized from the solvent mixture of methanol/water (1:1, v/v) to obtain 150mg of the desired compound (II).

25 The compound (II) thus obtained exhibits the following physico-chemical characteristics :

Chemical name : 3 β ,12 β -dihydroxy-damar-20(22),24-diene-3-
30 O- β -D-6"-O-acetyl-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside

Mass spectrum (FAB $^+$, m/z) : 809 ([M+H] $^+$), 831 ([M+Na] $^+$)

35 CNMR (δ ppm, pyridine-d5) : 13.1, 15.7, 15.9, 15.9, 17.1, 17.8, 18.5, 20.9, 25.7, 26.8, 27.1, 27.4, 28.1, 32.2, 32.6, 35.4, 37.1, 39.3, 39.8, 40.3, 50.8,

50.9, 51.1, 51.2, 56.5, 62.9, 64.8, 70.9, 71.1,
71.5, 75.4, 77.9, 78.1, 78.1, 78.6, 84.3, 89.2,
104.9, 106.2, 123.5, 125.2, 131.0, 140.8, 171.0

5 Example 5 : Synthesis of the compound (I)

50mg of ginsenoside Rg_3 was dried under reduced pressure and 1ml of 2,4,6-collidine was added thereto and then stirred for 10 minutes at -40°C. 10 μ l of acetyl chloride was introduced thereinto and then the mixture was allowed to react for 3 hours. The reaction mixture was warmed slowly to room temperature and allowed to stand for 1 hour at room temperature. 1ml of methanol was added and the reaction solution was subjected to silica gel column chromatography according to the same manner as Example 1 to obtain 20mg of the desired compound (I).

Example 6 : Synthesis of compound (II)

50mg of $\Delta^{20(22)}$ -ginsenoside Rg_3 was dried under reduced pressure and 1ml of 2,4,6-collidine was added thereto and then stirred for 10 minutes at -40°C. 10 μ l of acetyl chloride was introduced thereinto and then the mixture was allowed to react for 3 hours. The reaction mixture was warmed slowly to room temperature and allowed to stand for 1 hour at room temperature. 1ml of methanol was added and the reaction solution was subjected to silica gel column chromatography according to the same manner as Example 1 to obtain 25mg of the desired compound (II).

Test Example 1 : Anti-tumor activity of the compounds (I) and (II)

The anti-tumor activity of the novel saponin compounds of formulas (I) and (II) according to the present invention was determined by the method for measuring the incor-

poration amount of ^3H thymidine as described in the following.

13.8g of DMEM (Dulbecco's Modified Eagle's Medium, 5 manufactured by Gibco Co.) was dissolved in 1L of deionized water and then adjusted to pH 7.4 with sodium carbonate and hydrochloric acid solution. Then, 10% calf serum, $1\times 10^{-7}\text{M}$ of insulin and 50mg/L of gentamycin were added thereto. The mixture was then sterilized by means 10 of a millipore filter to prepare the culture solution. To this culture solution was inoculated human hepatoma sk-Hep-1 cell line, which was distributed from Cancer Research Center of Seoul National University in Korea, in a ratio of 1×10^6 cells per 25cm^2 of the T flask area, which 15 was then incubated for 48 hours in an incubator of 37°C while keeping 5% CO_2 gas. The culture product was transferred to a 24-well incubator and subcultured for one day, and then each of the compounds (I) and (II) dissolved in 70% ethanol was added thereto to a concentration of 0.01 20 to $10\mu\text{M}$, respectively. The same volume of 70% ethanol, instead of compounds (I) and (II), was added to the control group. 12 hours after treatment with each of the compounds (I) and (II), ^3H -labelled thymidine was added to a concentration of $1\mu\text{Ci}/\text{ml}$. After 12 hours, the medium 25 was removed from each well and the cells were fixed with methanol, washed with PBS and then washed twice with 10% trichloroacetic acid to remove the unreacted radioactive thymidine. The cells were dissolved in 1N sodium hydroxide solution and neutralized with 1N hydrochloric acid and 30 then the radioactivity introduced into DNA was measured by scintillation counter(Pharmacia 1024). The measured results are described in the following Table 1.

Table 1. Incorporation amount of radioactive thymidine into the DNA of hepatoma sk-Hep-1 cells depending on the concentration of compounds (I) and (II).

	Compound (I)			Compound (II)	
	Concentration (μ M)	dpm/well (Mean \pm S.E.)	Percentage to control	dpm/well (Mean \pm S.E.)	Percentage to control
Control	17812 \pm 819	100.0	25828 \pm 1918	100.0	
0.01	23281 \pm 1256	130.7	19242 \pm 570	74.5	
0.1	15016 \pm 1231	84.3	15658 \pm 1873	60.6	
0.5	15665 \pm 921	87.9	15137 \pm 1467	58.6	
1	15729 \pm 302	88.3	13646 \pm 2127	52.8	
2.5	13155 \pm 563	73.9	6421 \pm 1527	24.9	
5	733 \pm 145	4.1	430 \pm 105	1.7	
10	728 \pm 254	4.1	457 \pm 115	1.8	

20

Note S.E. : Standard error

As can be seen from the results described in Table 1 above, both the compounds (I) and (II) remarkably decrease 25 the amount of incorporated radioactive thymidine at a concentration of 0.1μ M or more, particularly 5μ M or more. Therefore, it can be seen that the compounds (I) and (II) significantly inhibit the growth of hepatoma sk-Hep-1 cells.

30

Test Example 2 : Cell growth-inhibitory activity of the compounds (I) and (II) against human hepatoma cells

35 13.8g of DMEM (Dulbecco's Modified Eagle's Medium, manufactured by Gibco Co.) was dissolved in 1L of deionized water and then adjusted to pH 7.4 with sodium carbon-

ate and hydrochloric acid solution. Then, 10% calf serum, 1×10^{-7} M of insulin and 50mg/L of gentamycin were added thereto. The mixture was then sterilized by means of a millipore filter to prepare the culture solution.

5 To this culture solution was inoculated human hepatoma cell line sk-Hep-1, which was distributed from Cancer Research Center of Seoul National University, in a ratio of 1×10^6 cells per 25cm^2 of the T flask area, which was then incubated for 48 hours in an incubator of 37°C while

10 keeping 5% of CO_2 gas. The culture product was transferred to a 96-well incubator in a concentration of 10^3 cells per well and subcultured for one day, and then each of the compounds (I) and (II) dissolved in 70% ethanol was added thereto to a concentration of 0.1 to $50\mu\text{M}$, respectively.

15 After 24 and 48 hours from the treatment with each of the compounds (I) and (II), $20\mu\text{l}$ of 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) dissolved in PBS(phosphate buffered saline) (5mg/ml) was added thereto and treated for 4 hours at 37°C to produce

20 the insoluble formazane. The reaction mixture was centrifuged and the supernatant was removed. Then, $100\mu\text{l}$ of DMSO(dimethylsulfoxide) was added to dissolve the formazane precipitate and then the optical density at 570nm was measured by means of automatic plate reader as an index of

25 the amount of formazane thus produced. The measured results are described in the following Tables 2 (after 24 hours) and 3 (after 48 hours).

Table 2. MTT analysis against human hepatoma sk-Hep-1 cells depending on the concentration of compounds (I) and (II) (after 24 hours)

Concentration (μ M)	Compound (I)		Compound (II)	
	O.D. (Mean \pm S.D.)	Percentage to control	O.D. (Mean \pm S.D.)	Percentage to control
Control	0.98 \pm 0.03	100.0	0.94 \pm 0.16	100.0
	0.88 \pm 0.05	89.7	0.70 \pm 0.02	74.6
	0.74 \pm 0.01	76.0	0.65 \pm 0.00	69.5
	0.68 \pm 0.05	69.6	0.59 \pm 0.04	62.7
	1	61.9	0.52 \pm 0.03	55.9
	5	50.7	0.46 \pm 0.04	48.7
	10	43.1	0.36 \pm 0.01	38.2
	25	17.3	0.24 \pm 0.03	26.0

Table 3. MTT analysis against human hepatoma sk-Hep-1 cells depending on the concentration of compounds (I) and (II) (after 48 hours)

Concentration (μ M)	Compound (I)		Compound (II)	
	O.D. (Mean \pm S.D.)	Percentage to control	O.D. (Mean \pm S.D.)	Percentage to control
Control	1.02 \pm 0.02	100.0	1.07 \pm 0.04	100.0
	0.89 \pm 0.04	87.8	0.90 \pm 0.04	83.8
	0.70 \pm 0.03	69.0	0.69 \pm 0.03	64.1
	0.42 \pm 0.02	41.6	0.42 \pm 0.01	38.7
	1	33.0	0.36 \pm 0.01	33.8
	5	18.1	0.28 \pm 0.04	26.0

Table 3. (continued)

5	Compound (I)			Compound (II)	
	Concentration (μ M)	O.D. (Mean \pm S.D.)	Percentage to control	O.D. (Mean \pm S.D.)	Percentage to control
10	10	0.14 \pm 0.01	13.7	0.14 \pm 0.01	12.7
	25	0.12 \pm 0.01	11.6	0.12 \pm 0.01	11.0

Note O.D. : Optical density
S.D. : Standard deviation

15

From the results described in Tables 2 and 3 above, it can be seen that since the optical density(O.D.) decreases accordingly as the concentration of the compound (I) or (II) increases, the compound of the present invention significantly inhibits the cell-growth of hepatoma sk-Hep-1 cells.

Test Example 3 : Acute toxicity test of the compounds (I)
25 and (II)

40 mice weighing 20 to 40g were used as test animal and divided into 2 groups including 20 mice, respectively. Each of the compounds (I) and (II) according to the present invention was suspended in 1ml of physiological saline and orally administered to each group. After 14 days from administration, the number of survived test animal was counted. To the control group, 1ml of physiological saline was orally administered. The results are described in the following Table 4.

Table 4. Acute toxicity of the compounds (I) and (II)
against mouse

5	Test group	Dosage (mg/kg, Oral)	Number of Test animals	Number of Survived animals
10	A	1000	20	20
	B	1000	20	20

Note A : Compound (I) receiving group
B : Compound (II) receiving group

15

From the results described in Table 4 above, it can be seen that the novel ginseng saponin compounds (I) and (II) according to the present invention have no substantial 20 toxicities.

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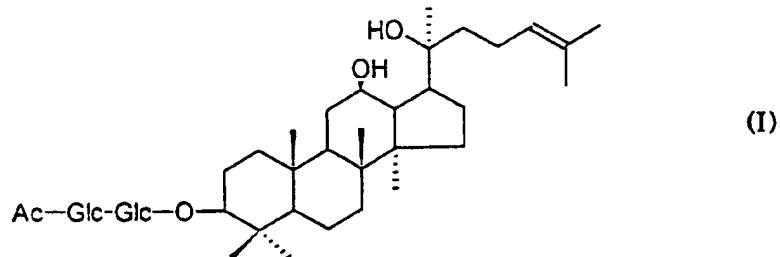
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WHAT IS CLAIMED IS :

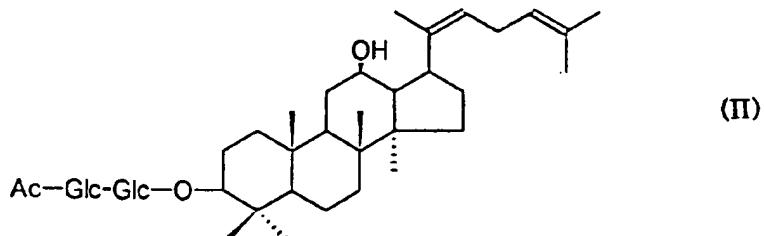
1. A ginseng saponin compound having the following formula
5 la (I):

10



15 2. A ginseng saponin compound having the following formula
la (II) wherein the configuration of $\Delta^{20}(22)$ is
zusammen or entgegen:

20

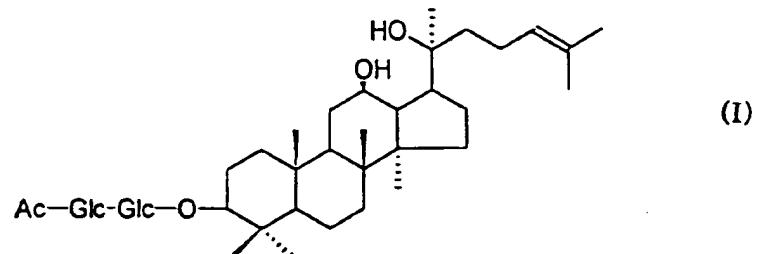


25

3. A process for preparing a compound having the following formula (I):

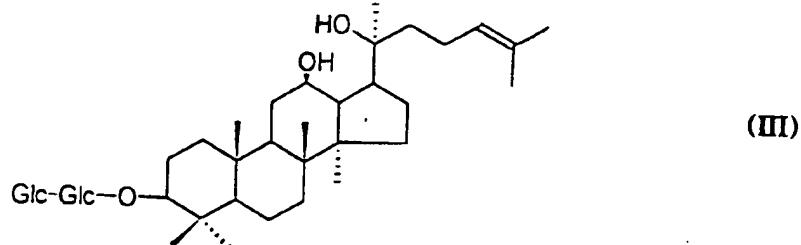
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which comprises acetyloyating ginsenoside Rg_3 having the following formula (III):

5

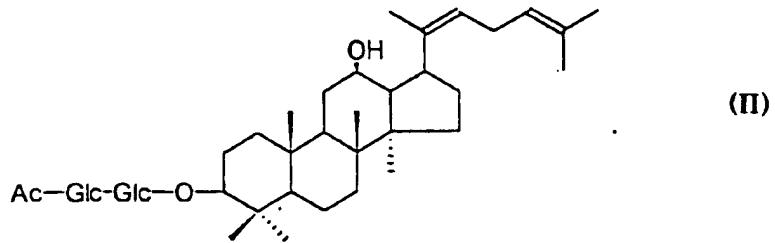


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4. A process for preparing a compound having the following formula (II):

15

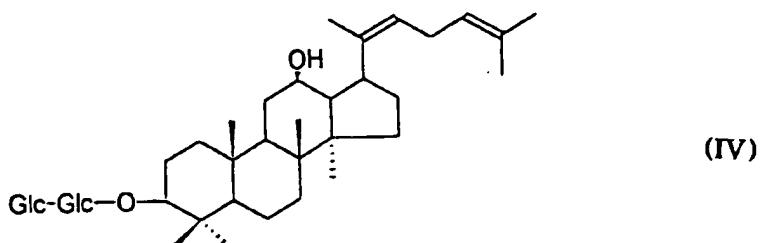
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25

which comprises acetyloyating $\Delta^{20(22)}$ -ginsenoside Rg_3 having the following formula (IV):

30



35 5. A process for preparing the compounds of formulas (I) and (II) as defined in claims 1 and 2, characterized in that an extract from the plant of Panax genus with

water or lower alcohol is heated for 0.5 to 20 hours at a temperature of 110 to 180°C, the processed ginseng thus obtained is extracted with water, an organic solvent or a mixture thereof and then the extract is concentrated under reduced pressure, suspended in water and then extracted with a nonpolar organic solvent, the aqueous layer is separated and extracted with a polar organic solvent, and the obtained extract is subjected to chromatography to obtain a fraction containing the said compounds (I) and (II), which is then crystallized from a solvent mixture of water and lower alcohol.

- 5 6. An anti-tumor composition comprising the compound of formula (I) as defined in claim 1, the compound of formula (II) as defined in claim 2 or a mixture thereof as an active component, together with a pharmaceutically acceptable carrier.
- 10 7. The composition of claim 6 which is formulated into a pharmaceutical dosage unit form.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR 96/00123

A. CLASSIFICATION OF SUBJECT MATTER

IPC⁶: C 07 J 17/00, 75/00; A 61 K 31/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC⁶: C 07 J 17/00, 75/00; A 61 K 31/705

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

QUESTEL: G-DARC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Chemical Abstracts, Vol.112, No. 19, 07 May 1990 (Columbus, Ohio, USA), page 432, abstract No. 175597h, I.KITAGAWA et al., "Chemical studies on crude drug processing. VI. Chemical structures of malonyl-ginsenosides Rb1, Rb2, Rc and Rd isolated from the root of Panax ginseng C.A. Meyer", & Chem.Pharm.Bull. 1989, 37(11) 2961-70.	1,5
A	Chemical Abstracts, Vol.123, No.9, 28 August 1995 (Columbus, Ohio, USA), page 748, abstract No. 122844g, D.S.KIM et al., "Preparation and structur determination of a new glycoside, (20E)-ginsenoside Rh3, and its isomer from diol-type ginseng saponins.", & Yakhak Hoechi 1995, 39(1) 85-93.	2,5
A	Chemical Abstracts, Vol.107, No.3, 20 July 1987 (Columbus, Ohio, USA), page 642, abstract No. 23596r, L.N.ATOPKINA et al., "Glycosylation of dammarane type triterpenoids. IV. B-D-Glucopyranosides of betulafolienetriol and its derivatives", & Khim. Prir. Soedin. 1968, (3), 301-12.	1

 Further documents are listed in the continuation of Box C. See patent family annex.

- Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

16 January 1997 (16.01.97)

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR 96/00123

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 40 01 895 A1 (HARRIER GMBH) 25 July 1991 (25.07.91), pages 1-7. -----	2,6,7

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/KR 96/00123

In Recherchenbericht angeführtes Patentdokument Patent document cited in search report	Datum der Veröffentlichung Publication date	Mitglied(er) der Patentfamilie Patent family member(s)	Datum der Veröffentlichung Publication date	
Document de brevet cité dans le rapport de recherche	Date de publication	Membre(s) de la famille de brevets	Date de publication	
DE A1 4001895	25-07-91	AU A1 71489/91 CA AA 207369/91 CN A 105323/91 EP A1 59458/91 HU A0 920212/91 HU A2 61778/91 IL A0 96983/91 IL A1 96983/91 JP T2 550454/91 JP A2 810918/91 US A 527829/91 US A 549680/91 WO A1 911145/91 AU A1 240117/91 CA AA 211381/91 CN A 107317/91 CN B 103164/91 EP A1 597010/91 EP A4 597010/91 IL A0 102566/91 JP T2 680145/91 WO A1 930182/91	AU A1 71489/91 CA AA 207369/91 CN A 105323/91 EP A1 59458/91 HU A0 920212/91 HU A2 61778/91 IL A0 96983/91 IL A1 96983/91 JP T2 550454/91 JP A2 810918/91 US A 527829/91 US A 549680/91 WO A1 911145/91 AU A1 240117/91 CA AA 211381/91 CN A 107317/91 CN B 103164/91 EP A1 597010/91 EP A4 597010/91 IL A0 102566/91 JP T2 680145/91 WO A1 930182/91	21-08-91 24-07-91 24-07-91 04-05-94 28-12-92 01-03-93 29-03-93 21-03-93 15-07-93 30-04-94 11-01-94 06-03-94 08-08-91 22-02-93 04-02-93 16-06-93 24-04-96 18-05-94 07-02-96 14-01-93 17-02-94 04-02-93